

Effects of Wavelengths of Light on the Photoperiodic Gonadal Response of Blinded-Pinealectomized Japanese Quail

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ABSTRACT—Action spectra for the photoperiodic gonadal response of blinded-pinealectomized quail were investigated by night interruption experiments in which light pulses (1 hour) with various wavelengths were given at the photoinducible phase, 13 hours after the onset of main photoperiod (8L16D). Spectral transmittance of the brain tissues was measured and the percent transmittance at the hypothalamus was obtained. Long wavelength light (750 nm) penetrates about 650 times more than short wavelength light (450 nm). The difference spectra between the transmittance measured at the dorsal and the ventral borders of the hypothalamus represents the absorption by the hypothalamic tissues and showed two peaks at 500 and 600 nm. Action spectra measured by equalizing the number of photons reaching the hypothalamus showed a peak at 500 nm for the photoperiodic responses of the testis and the cloacal gland in blinded-pinealectomized quail. Since the light pulse delivered at the photoinducible phase was effective to induce gonadal growth in blinded-pinealectomized quail, the circadian oscillator as well as the photoreceptor for the photoperiodic time measurement seems to be located somewhere other than the eye and the pineal.

INTRODUCTION

The importance of extraocular photoreception for the photoperiodic gonadal response (gonadal development induced by long photoperiods) in birds has been known since Benoit's pioneer work in 1935 [2, 19, 24]. The encephalic photoreceptor seems to be the most important extraocular photoreceptor [3, 7, 22], although the pineal organ might also be involved as an extraocular photoreceptor in the photoperiodic gonadal response of Japanese quail [21] and ducks [14].

In our previous paper [23], we reported a stimulative effect of red light on the maintenance of testicular size in Japanese quail, when light intensity was adjusted at the bird height. This effect of red light was confirmed in quail [5] and cockerels [27] by measuring plasma luteinizing hormone and testosterone concentration. However, direct stimulation of the presumed encephalic photoreceptors by implantation of radioluminescent substance

[15, 25] or light-conducting fibers [3, 12, 35] demonstrated that these photoreceptors are sensitive to a broad range of wavelengths within the visible light. Recently, Foster and his colleagues [7, 8] illuminated the hypothalamic region with equal numbers of photons at a range of wavelengths via a fiber optic placed on the skull, and suggested that the photopigment of the encephalic photoreceptor involved in the photoperiodic response of quail is a rhodopsin-like substance because the action spectrum for the response indicated a very similar pattern to the absorption spectrum of rhodopsin. However, since the pineal was kept intact in their experiment, they failed to distinguish whether the response to light was through the encephalic photoreceptor or through the pineal that is a possible photoreceptor for the photoperiodic gonadal response [14, 21] and circadian rhythms [9] and has a photopigment immunoreactive to the antiserum against rhodopsin [1, 4, 18, 32].

In the present study, we measured transmittance of various wavelengths of light through overlying tissues into the hypothalamus in decapitated heads of intact and blinded-pinealectomized Japanese quail. We, then, adjusted the number of photons

Accepted June 18, 1993

Received March 19, 1993

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delivered to the dorsal border of the hypothalamus to become equal for each wavelength in blinded-pinealectomized quail, and recorded the action spectra for gonadal development by inserting one-hour pulses of monochromatic light at the photoinducible phase. "Photoinducible phase" is so called because a light pulse interrupting the dark period at a certain phase induces gonadal development even though the animals are under short photoperiods, and the circadian time measurement system is considered to be involved [6, 29, 33].

MATERIALS AND METHODS

Japanese quail (*Coturnix coturnix japonica*) were obtained from a local breeder. Food and water were given *ad libitum*. The large spectrograph in National Institute for Basic Biology, Okazaki, Japan, was used to obtain monochromatic light of short band width. Details of the spectrograph design were reported by Watanabe *et al.* [34]. A 30 kW xenon short arc lamp was used in Exp. 1 and a 6 kW lamp in Exp. 2. Intensity of light was measured by UDT 181 radiometer (United Detector Technology Co., Culver City, California) and YSI 65A radiometer (Yellow Spring Instruments, Yellow Springs, Ohio).

Exp. 1 Spectral transmittance of the brain tissues

A head without lower jaw (3 intact and 3 blinded-pinealectomized) of 5-week old quail just after decapitation was placed in a black plastic box with two holes (5 mm in diameter). Monochroma-

tic light (from Okazaki large spectrograph, band width 0.5 nm) was introduced via the upper hole and a photo-detector (UDT 181) was placed at the bottom hole. Stray light was omitted by covering the apparatus by a black rubber sheet with a hole on the top. Intensity of light was measured (1) without the head (blank), (2) in the dorsal midline just beneath the skull, (3) at the dorsal border of the hypothalamus, and (4) at the ventral border to the hypothalamus, and percent transmittance against the blank was plotted for each case. Absorption by the hypothalamus was shown as the difference between the percent transmittance at the dorsal and the ventral borders of the hypothalamus.

Exp. 2 Action spectra for the photoperiodic gonadal response in blinded-pinealectomized quail

Thirty quail (4 week-old) reared under 8L16D (light period: 03:00–11:00) were pinealectomized and bilaterally enucleated under Nembutal anesthesia. One week later, birds were housed in individual cages and divided into 7 groups assigned to several wavelengths. One group was maintained under 8L16D and served as control. The other groups were also maintained in 8L16D, but they received in addition one-hour pulses of monochromatic light at 13 hours (16:00–17:00; photoinducible phase) after the onset of main photoperiod. Therefore, the lighting regimen was 8L:5D:1L:10D, and the birds were maintained in this lighting regimen for 15 days. To provide the monochromatic light, birds were taken out from

TABLE 1. Intensity of light at various wavelengths. The intensity of light at the surface of the head was adjusted so as to give the same amount of photons at the dorsal border of hypothalamus for each monochromatic light

Wavelength (nm)	Intensity of light ($\mu\text{W}/\text{cm}^2$)		No. of photons/ cm^2/sec Hypothalamic level
	Surface of the head	Hypothalamic level	
		$\times 10^{-4}$	$\times 10^9$
750	0.32	4.34	1.63
650	1.04	5.41	1.76
600	5.0	5.55	1.67
550	27.0	5.54	1.52
500	18.0	7.11	1.78
450	135	7.16	1.61

their individual cages, and immobilized on a board, and were placed in a light-proof box. All of these procedures were carried out in complete darkness. And then, these light-proof boxes were brought into the spectrograph room and the birds were illuminated by removing the lids of the boxes. Wavelengths chosen were 450, 500, 550, 600, 650 and 750 nm. The intensity of light was adjusted by neutral density filters so as to give the equal number of photons at the dorsal border of the hypothalamus for each monochromatic light (Table 1), and it was almost the same as that reported by Foster and Follett [7]. At 5, 8, 11 and 15 days after the beginning of the experiment, the size of the cloacal gland protrusion (the secondary sex characteristic of males) was measured with a caliper, and, at the end of the experiment, birds were sacrificed by decapitation and testes weight was measured. Completeness of pinealectomy was checked anatomically. Statistical analysis was done by Duncan's multiple range test.

RESULTS

The result of Exp. 1 is shown in Fig. 1. The percent transmittance under the skull against the blank decreased gradually from 750 nm to 500 nm and drastically from 500 nm to 350 nm in the head of intact quail. In the case of blinded-pinealectomized quail, the reduced transmittance was observed in the whole range of visible spectrum due to suturing of the skin. At the dorsal border of the hypothalamus of intact and blinded-pinealectomized quail, the percent transmittance decreased gradually from 750 nm to 650 nm and then drastically to 550 nm. It increased again at 500 nm. At 450 nm, the minimum transmittance was observed. The difference spectra between the transmittance measured at the dorsal and the ventral borders of the hypothalamus represent the absorption of the hypothalamic tissues and showed two peaks at 500 and 600 nm.

The results of Exp. 2 are shown in Fig. 2. When light-pulses were inserted at the photoinducible phase (13 hours after the onset of main photoperiod) for 15 days, testicular weight at 500 nm increased significantly (0.294 ± 0.109 g), while those at 450, 650 nm and control remained small

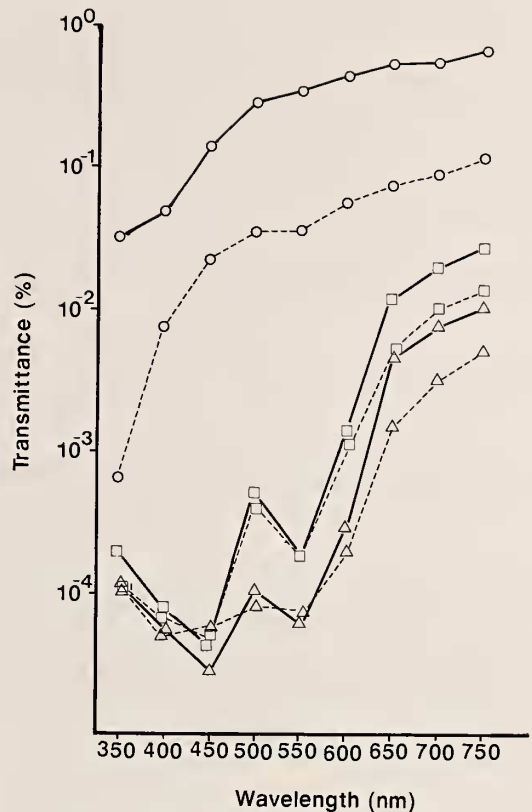


FIG. 1. Transmittance (%) of the brain tissues for various wavelengths. Circles, squares and triangles indicate transmittance under the skull, at the dorsal border and at the ventral border of the hypothalamus, respectively. Solid and broken lines indicate transmittance measured in intact and blinded-pinealectomized birds, respectively.

(0.046 ± 0.032 g, 0.031 ± 0.010 g and 0.079 ± 0.030 g, respectively). The difference between the values at 500 nm and 650 nm was statistically significant ($P < 0.05$). The values at 550 and 600 nm were intermediate. The response of the cloacal gland was more distinct. The cloacal gland growth could be detected at 11 days after the beginning of the experiment. At the end of experiment, the value at 500 nm (0.693 ± 0.118 cm²) was significantly larger than those at 600 nm (0.305 ± 0.027 cm²) and 650 nm (0.325 ± 0.029 cm²) ($P < 0.05$). The value at 500 nm was not significantly different from that of control (0.393 ± 0.076 cm²), but it was close to the significant level. The peak at 500 nm coincided with one of the absorption peaks of

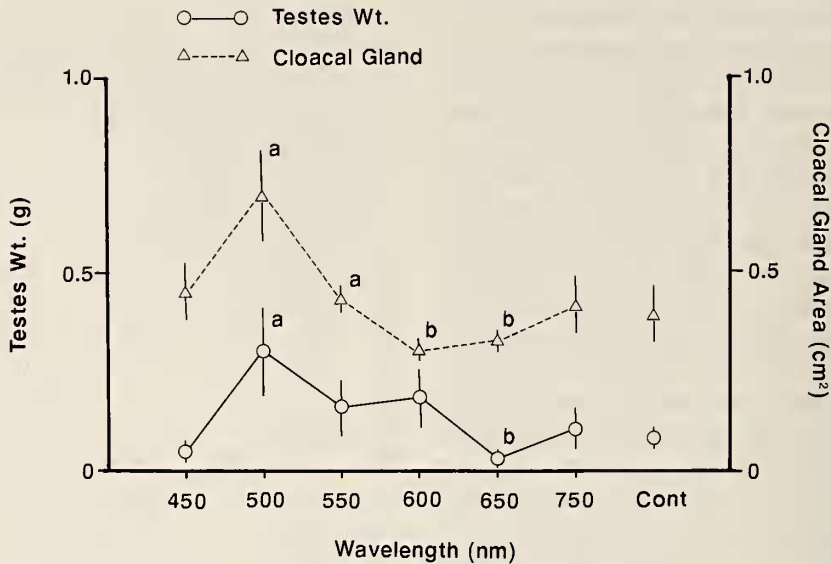


FIG. 2. Effects of one-hour light pulses with various wavelengths on the growth of the testis and cloacal gland in blinded-pinealectomized Japanese quail. The light pulses were inserted at the photoinducible phase, 13 hours after the onset of main photoperiod (therefore, lighting regimen is 8L:5D:1L:10D). The intensity of light was adjusted so as to give equal numbers of photons at the dorsal border of the hypothalamus at a range of visible spectrum. Testicular weight and cloacal gland size after 15 days of lighting treatment were shown. Significant difference between a and b: $P < 0.05$.

hypothalamic tissues. These results clearly indicate that photoperiodic responses of the testis and cloacal gland were induced via the extraocular and extrapineal photoreceptor, and suggest that the site of the circadian oscillator for the photoperiodic time measurement is somewhere other than the eye and pineal.

DISCUSSION

The results on spectral transmittance of brain tissues in intact Japanese quail confirmed the reports by Hartwig and Van Veen [13] and Foster and Follett [7]. Since the spectral transmittance of brain tissues in blinded-pinealectomized quail was similar to that in intact quail, suturing of the skin after operation did not change the basic pattern of the spectral transmittance.

The results obtained from the present action spectrum experiment in blinded-pinealectomized quail by equalizing the number of photons reaching the hypothalamus for each wavelength confirmed those in the photoperiodic responses of

intact quail reported by Foster and his colleagues [7, 8], and were different from those of previous studies in which light intensities were adjusted to equal energy at the surface of the head [23]. Discrepancy between the previous studies (red light was more effective [23]) and the present study is probably due to the fact that the light with longer wavelengths penetrate through the brain tissues more than that with shorter wavelengths. Although the results using intact quail could not distinguish the effects via the hypothalamic photoreceptor from the effects via the pineal [14, 21] which has photoreceptor-like structures [20] and a rhodopsin-like photopigment [1, 4, 10, 18, 32], the present study in blinded-pinealectomized quail clearly demonstrated the role of the encephalic photoreceptor in the photoperiodic gonadal response. The peak of the photoperiodic response at 500 nm coincided well with the absorption peak of hypothalamic tissues and suggests that a rhodopsin-like pigment in the hypothalamus is involved in the photoperiodic gonadal response of quail. By the use of antiserum against rhodopsin [16], we

obtained preliminary results on immunopositive cells in the hypothalamus of quail and Japanese grass lizards (Oishi *et al.*, unpublished) similar to the reports by Silver *et al.* [28] in birds and Foster *et al.* [11] in lizards.

Since the light pulse inserted at the photoinducible phase was effective to induce photoperiodic response of gonads, the present study suggests that a circadian clock system is involved in the photoperiodic time measurement as reported by several authors [6, 29, 33]. Furthermore, since our experiment was performed in blinded-pinealectomized quail, the location of the circadian oscillator for the photoperiodic time measurement as well as the circadian photoreceptor is somewhere other than the eye and pineal, probably the hypothalamus. Therefore, the site of the oscillator for the photoperiodic time measurement seems to be different from the site for the locomotor activity rhythm in quail (the eye) as reported by Underwood and his colleagues [30, 31] and Konishi *et al.* [17]. This is in agreement with the report by Follett *et al.* [6] who suggested different oscillator systems for rhythms of "photoinducibility" and locomotor activity in Japanese quail.

ACKNOWLEDGMENTS

We are grateful to Dr. M. Watanabe, National Institute for Basic Biology, for his help and encouragement throughout this research, and Dr. A. Masuda for critical reading of the manuscript. This work was partly supported by a grant 81A03 for researches at Okazaki Large Spectrograph, National Institute for Basic Biology.

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